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14. ABSTRACT
In this funding cycle, we concentrated on encapsulating NZ51 into nanoparticles that are biocompatible and biodegradable. Earlier, we had reported that we were successful in preparing PLGA nanoparticles loaded with GdDTPA-BOA and superparamagnetic iron oxide (SPIO) nanoparticles. Based on that technology, we initiated experiments to load PLGA nanoparticles with NZ51. After a series of experiments, we concluded that there was significant variation from batch to batch preparation and we have to refine the technology used. Currently, we are attempting to encapsulate the RNA helicase inhibitor into a different nanoparticle, such as PLGA nanoparticles composed of low molecular weight copolymers, or nanoparticles composed of chitosan derivatives, with the goals of achieving a faster release resulting in increased efficacy for breast cancer treatment. Also, we are in the process to determine why the different batches made have different aggregation properties in mouse plasma. Furthermore, we are attempting to encapsulate a different DDX3 inhibitor into PLGA nanoparticle to evaluate if the chemical structure of NZ51 is in anyway interfering with the formulation and utility in in vivo experiments.

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Introduction

Understanding tumor invasion and metastasis provides crucial information with respect to carcinoma progression. Towards this goal, we have identified a member of the RNA helicase family, *DDX3*, which is over-expressed in high-grade invasive breast carcinomas and induces an epithelial mesenchymal-like transformation with increased motility and invasive properties (Botlagunta, 2008). More importantly, decreasing DDX3 expression by shRNA reduced the metastatic load in a preclinical breast cancer model. Based on this crucial finding, we evaluated the efficacy of a novel DDX3 inhibitor, NZ51, to potentiate cell death or reduce proliferation in breast cancer cell lines (MCF-7, MDA-MB-468 and MDA-MB-231) but not in normal immortalized breast cell lines (MCF 10A and MCF 12A). The NZ51 was designed using rational molecular modeling approach to bind to the nucleotide binding site within the DDX3 protein molecule and abrogate its function. Preliminary data strongly indicates that NZ51 is able to selectively induce cell death in the panel of breast cancer cell lines used and not in normal immortalized breast cell lines. The above results demonstrate that abrogating DDX3 functions in breast cancer cell lines, irrespective of ER status, can promote cell death-a mechanism that can be targeted to overcome treatment inadequacies for aggressive breast cancer such as that of the triple negative phenotype.

In this study, we proposed to study the therapeutic impact of NZ51, both in the native form as well as in nanoparticles containing dual-MR contrast agent, on the primary orthotopic tumor in a preclinical breast cancer model using non-invasive magnetic imaging techniques. Our ultimate goal is to provide targeted therapy for aggressive breast cancer phenotypes with longer disease free survival period and a better quality of life.

Body

Task 1: Generating NZ51-loaded PLGA nanoparticles

The ultimate goal of this proposal is to translate our novel RNA helicase inhibitor, NZ51, into clinical applications. Due to its low water-solubility, one of the best ways to achieve such a goal is to encapsulate NZ51 into nanoparticles that are biocompatible and biodegradable. Nanoparticles allow predominant accumulation of cargo agents in the tumor where the vasculature is tortuous and leaky and the lymphatic drainage is dysfunctional. This phenomenon is called "enhanced permeability and retention (EPR) effects" (Matsumura, 1986), and this is an essential part of passive targeting strategy, and also plays a pivotal role in active targeting strategy. We have chosen poly-(D,L-lactide-co-glycolide) (PLGA) as a composition of nanoparticles because this co-polymer is already used for biodegradable surgical sutures and sustained drug release formulations in clinic. As we showed in the last progress report, we successfully prepared PLGA nanoparticles, and characterized *in vitro* release properties with MRI by incorporating GdDTPA-BOA and superparamagnetic iron oxide (SPIO) nanoparticles. As held in many laboratories, we used a solvent evaporation method, which is more suitable for laboratory small-scale production. One of the obstacles to clinical translation of nanotherapeutics is mass production, which often leads to non-uniformity of the formulations (K. Farahani, personal communication). In fact, although we could prepare 5-fluorouracil-loaded PLGA nanoparticles consistently (Onuki, 2010), the variability from batch to batch was relatively large in the case of PLGA nanoparticles loaded with an RNA helicase inhibitor (**Table 1**).

Table 1. Physicochemical characteristics of PLGA nanoparticles loaded with or without a novel RNA helicase inhibitors prepared by a solvent evaporation method.

Batch #	Hydrodynamic diameter	Polydispersity index	ζ-potential	Encapsulation efficiency	Aggregation in mouse plasma
1	197 nm	0.068	-2.39 mV	14.6%	No
2	186 nm	0.168	-1.63 mV	—	No
3	212 nm	0.103	-2.11 mV	32.7%	Yes
4	233 nm	0.184	-1.84 mV	47.6%	Yes
5	217 nm	0.123	-3.20 mV	—	Yes
6	262 nm	0.271	-4.00 mV	48.0%	No

Task 2: Generating different types of nanoparticles for faster degradation in physiological conditions

To accelerate clinical translation of our nanotherapeutics, we need to consider switching preparation method suitable for a large-scale production, for example, a spray drying method (**Figure 1**). This method enables us to prepare relatively uniform particles with a large-scale. In addition, the release kinetics of the RNA helicase inhibitor from the generated nanoparticles is extremely slow and a large variability between batches making it less effective for cancer treatment. To resolve this we are attempting to use different composition of nanoparticles, which degrades faster in physiological conditions. Namely, we will attempt to encapsulate the RNA helicase inhibitor into a different nanoparticle, such as PLGA nanoparticles composed of low molecular weight copolymers, or nanoparticles composed of chitosan derivatives, with the goals of achieving a faster release resulting in increased efficacy for breast cancer treatment.

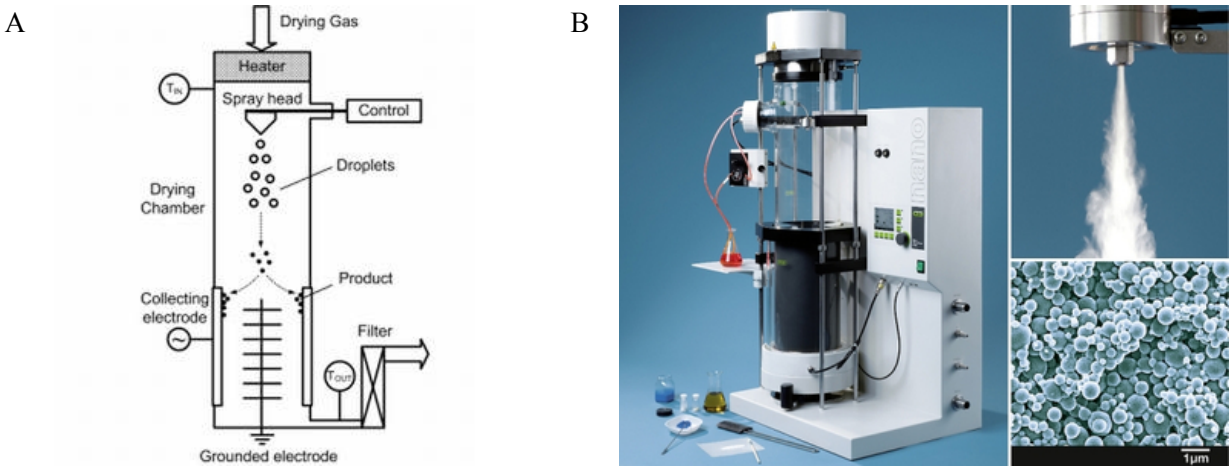

Figure 1. Illustrations of Nano spray dryer. (A) Functional principle of a nano spray dryer. (B) Nano spray dryer B-90. Adapted from www.buchi.com.

Task 3: Encapsulating different RNA helicase inhibitors into nanoparticles

An increase in the hydrophilicity of the agent is another example to promote achievement of our ultimate goal, clinical translation. Hydrophilic agents are generally easy to handle compared to hydrophobic agents. The best example is doxorubicin that is used clinically as a hydrochloride salt (doxorubicin hydrochloride). We can prepare nanotherapeutics using hydrophilic agents as with Doxil® that is a liposomal formulation of doxorubicin hydrochloride. Similarly, even if we change the agent from NZ-51 to other RNA helicase inhibitors, we will be able to develop nanotherapeutics, such as drug-loaded polymeric nanoparticles or liposomes. The RNA helicase inhibitors used in our ongoing projects are shown in **Figure 2**.

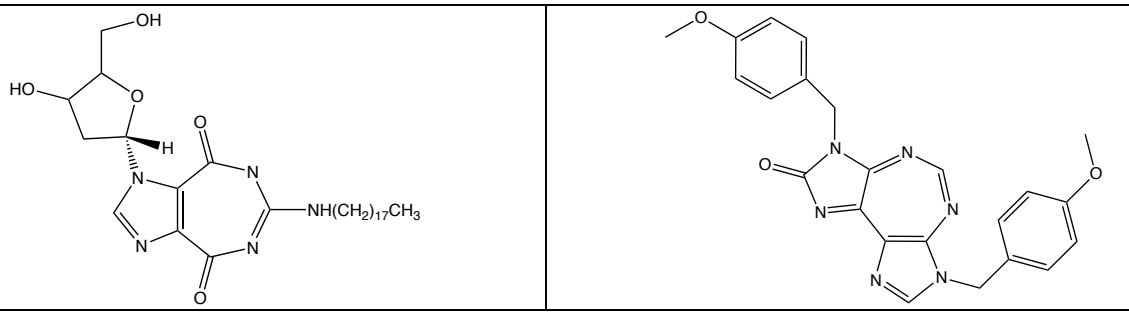

Figure 2. Chemical structures of other RNA helicase inhibitors that we are currently working on. (A) NZ51; (B) RK-33

Key research accomplishments

1) Generated and characterized PLGA nanoparticles loaded with NZ51.
2) Determined that the generated NZ51-loaded PLGA nanoparticles have a hydrodynamic diameter of about 200 nm and with a slightly negative surface charge (≈-2.5 mV).
3) Release kinetics experiments indicate that NZ51 has a slow release rate in 10% mouse plasma (pH 7.4) over 24 hr period.

Reportable outcome

None

Conclusions

Based on the work we have carried out, it appears that the technology we used to generate the nanoparticles is not optimal due to the inconsistency in the encapsulation efficiency as well as the release kinetics in mouse plasma. Due to the technical difficulties faced, we are now generating nanoparticles using different substrates. In addition, we are evaluating other RNA helicase inhibitor to rule out any interference from the chemical structure of NZ51 that could possibly impede the generation of the nanoparticle.

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